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Biosynthesis of Stress Ethylene in Soybean Seedlings: Similarities to Endogenous Ethylene Biosynthesis

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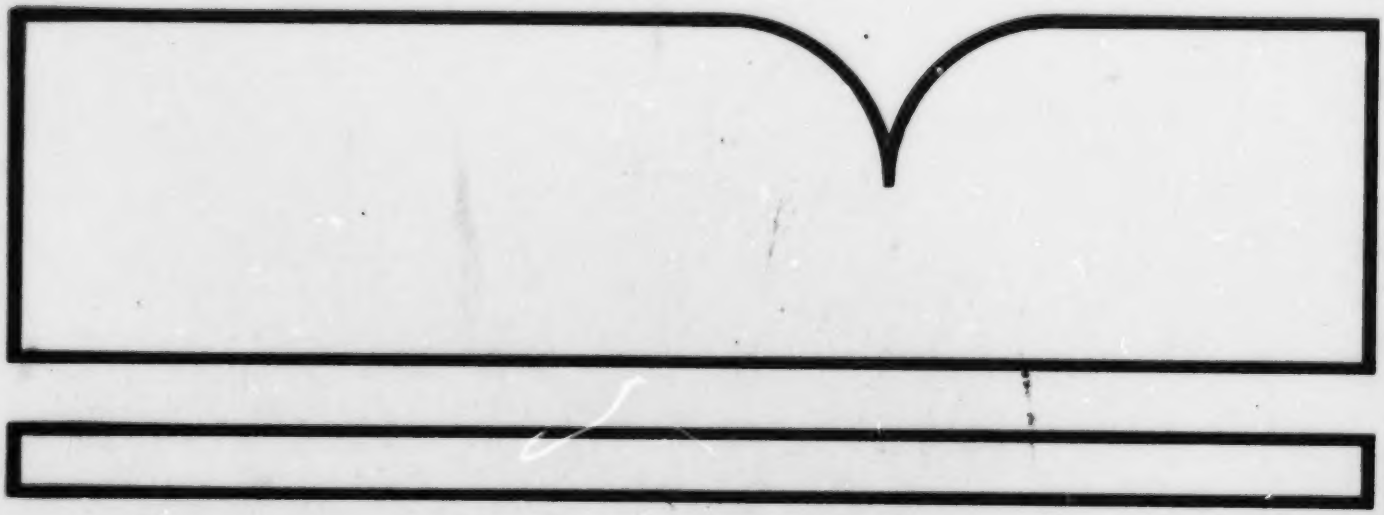

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16. ABSTRACT

The similarity of stress ethylene biosynthesis in whole plants to endogenous ethylene biosynthesis was investigated using two inhibitors of ethylene biosynthesis, aminoethoxyvinylglycine (AVG) and cobalt chloride (Co^{2+}); and the intermediates, methionine, S-adenosylmethionine (SAM), and 1-aminocyclopropane-1-carboxylic acid (ACC), of basal ethylene biosynthesis. Stress ethylene production induced by ozone, cadmium, or 2,4-dichlorophenoxyacetic acid was inhibited in hydroponically-grown soybean seedlings in a concentration-dependent manner by both AVG and CO^{2+}. The ethylene intermediates evoked responses in intact seedlings similar to that described for endogenous ethylene production in isolated vegetative tissue. The addition of SAM to the hydroponic system relieved AVG inhibition of stress ethylene production. Feeding ACC to the seedlings resulted in increased ethylene production independent of stress application or prior AVG inhibition. Cobalt inhibition of stress ethylene production was relieved by increasing concentrations of ACC. A short lag period of 12-18 min was observed in stress ethylene production following a 30-min ozone exposure. Addition of cycloheximide partially inhibited ozone-induced ethylene production. These results suggest a common pathway in whole plants for stress ethylene production and endogenous ethylene biosynthesis.

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Biosynthesis of stress ethylene in soybean seedlings: Similarities to endogenous ethylene biosynthesis

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The similarity of stress ethylene biosynthesis in whole plants to endogenous ethylene biosynthesis was investigated using two inhibitors of ethylene biosynthesis, aminoethoxyvinylglycine (AVG) and cobalt chloride (Co^{2+}); and the intermediates, methionine, *S*-adenosylmethionine (SAM), and 1-aminocyclopropane-1-carboxylic acid (ACC), of basal ethylene biosynthesis. Stress ethylene production induced by ozone, cadmium, or 2,4-dichlorophenoxyacetic acid was inhibited in hydroponically-grown soybean seedlings in a concentration-dependent manner by both AVG and CO^{2+}. The ethylene intermediates evoked responses in intact seedlings similar to that described for endogenous ethylene production in isolated vegetative tissue. The addition of SAM to the hydroponic system relieved AVG inhibition of stress ethylene production. Feeding ACC to the seedlings resulted in increased ethylene production independent of stress application or prior AVG inhibition. Cobalt inhibition of stress ethylene production was relieved by increasing concentrations of ACC. A short lag period of 12–18 min was observed in stress ethylene production following a 30-min ozone exposure. Addition of cycloheximide partially inhibited ozone-induced ethylene production.
These results suggest a common pathway in whole plants for stress ethylene production and endogenous ethylene biosynthesis.

Additional key-words: Stress ethylene, ozone, aminoethoxyvinylglycine (AVG), cobalt, l-aminocyclopropane-l-carboxylic acid (ACC), soybean, *Glycine max*, hydroponics.

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Introduction

Ethylene production increases significantly above basal levels in plant tissues following a variety of stresses including mechanical wounding, drought, chilling, freezing, water-logging, irradiation, and pathogenic organisms (Abeles 1973, Yang and Pratt 1978, Leiberman 1979). Contact with phytotoxic chemicals including cadmium, copper, Endothal, phytar 560 and ozone also increases stress ethylene production by whole plants (Craker 1971, Tingey 1980) and plant tissues (Abeles and Abeles 1971).

The biosynthesis and regulation of endogenous ethylene has been more intensely studied than stress ethylene (Leiberman 1979). In isolated vegetative and fruit tissue the endogenous ethylene precursor is methionine and the production is regulated by internal levels of auxin (Abeles 1973, Leiberman 1979, Yu and Yang 1979). The pathway for endogenous and auxin-induced biosynthesis in several vegetative and fruit tissues has recently been described as methionine → SAM → ACC → ethylene (Adams and Yang 1979, Boller *et al.* 1979, Jones and Kende 1979, Yu and Yang 1979). In these studies, two ethylene inhibitors, AVG and cobalt, blocking two distinct sites, were useful in elucidation of the biosynthetic sequences. AVG inhibits the

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conversion of SAM → ACC in a variety of tissues (Leiberman *et al.* 1975, Yu and Yang 1979, Jones and Kende 1979). Cobalt prevents the oxidation of ACC → ethylene (Yu and Yang 1979).

There are few studies of the biosynthesis of stress-induced ethylene in plant tissues. In a ^{14}C study with bean leaves, Abeles and Abeles (1972) suggested methionine as the precursor of stress-induced ethylene. However, the conversion efficiency of ^{14}C-methionine to ^{14}C-ethylene was quite low, suggesting the possibility of another pathway. Additional evidence for methionine as the precursor of stress ethylene is provided by the inhibition of wound ethylene production with the ethylene inhibitor AVG in isolated flower petal tissue (Kende and Hanson 1976), and etiolated pea stem sections (Saltveit and Dilley 1979). Yu and Yang (1980) recently demonstrated not only the inhibition of wound ethylene production, but also the synthesis of ACC and ACC synthase activity with AVG in mung bean hypocotyl and unripe tomato tissue.

The objective of the present study was to investigate the similarities of stress ethylene production to endogenous ethylene synthesis in order to validate the biochemical basis of stress ethylene bioassays employed in phytotoxicant studies. Intact, actively growing seedlings were utilized, in contrast with previous studies which employed isolated vegetative or fruit tissue. A broad range of stresses was examined in conjunction with two known inhibitors of endogenous ethylene synthesis; and the biosynthetic intermediates of ethylene synthesis were tested for their ability to overcome inhibition. In addition, the regulation of stress ethylene production in whole plants was examined by a time course study of ethylene production during the first 2 h following ozone exposure and the effect of cycloheximide on this stress ethylene production.

Abbreviations: SAM, *S*-adenosylmethionine; ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine; Co^{2+}, cobalt chloride; 2,4-D, 2,4-dichlorophenoxyacetic acid.

Materials and methods

Plant material and culture

Soybean seeds, *Glycine max* L. cv. Dare, were sown in vermiculite and seedlings grown under a 16-h photoperiod (325–425 μE m^{-2} s^{-1}) in a controlled environment chamber with day/night temperatures of 26–21°C ± 1°C and relative humidity of 27–31%. After 6 days, each seedling was transferred to a continuously aerated hydroponic system consisting of a 250-ml polypropylene nutrient container with a lid designed to hold the seedling upright and permit encapsulation (Fig. 1). North Carolina State University Phytotron nutrient solution (Downs and Hellmers 1975) was supplied as required to replace transpirational loss; the nutrient was changed 3 days after seedlings were transferred. Four days after transfer, the seedlings (10 days old) were exposed to various ethylene synthesis inhibitors, intermediates and stresses.

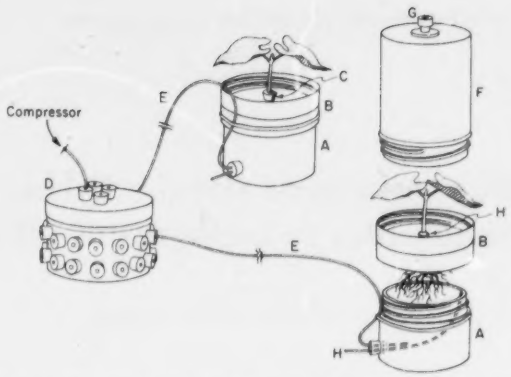

Fig. 1. Hydroponic system for growth, stress exposure and ethylene determination. 5–7 day old soybean seedlings were placed in the 250-ml silvered polypropylene nutrient container (A) and, held upright in place through a 1-cm hole in specially adapted lids (B). The stems are secured with polyurethane foam (C) during growth and stress exposure. Constant aeration is delivered from compressor through a manifold (D) to the individual hydroponic container via 0.2 cm polyethylene line (E). For ethylene determinations the plants were usually encapsulated with a 500-ml polypropylene container (F) equipped with septum (G) for head space air sampling. Modeling clay (H) is used to seal off root container (A) during encapsulation.

Inhibitors

The ethylene synthesis inhibitors AVG, Co^{+2} or cycloheximide were introduced to the hydroponic system at known concentrations in fresh nutrient at various time intervals before exposure of the seedlings to stress.

Ethylene intermediates

The seedlings received 1.0 μ*M* AVG in the hydroponic nutrient for 2 h prior to ozone exposure. At the time of ozone exposure, the nutrient was replaced with fresh media containing either 1.0 m*M* methionine, 1.0 m*M* SAM or 0.5 m*M* ACC to test the ability of these intermediates to overcome AVG inhibition. The seedlings were exposed to 0.6 μl l^{-1} ozone for 2 h, encapsulated and incubated in the dark for 1 h at 26°C prior to ethylene determinations. Previous studies have shown an increased stress ethylene production with dark incubation (Tingey *et al.* 1976).

The ability of ACC to overcome Co^{2+}-inhibited ozone-induced ethylene was assessed after treating seedlings in nutrient containing 10 m*M* $CoCl_2$ 2 h prior to ozone exposure. At the time of exposure, the nutrient was replaced with nutrient containing 200 μ*M* ACC. ACC concentration effects on Co^{2+} inhibition of basal

ethylene was also examined. The seedlings were placed in nutrient containing 10 m*M* CoC_2 for 2 h. Following this incubation, the nutrient was replaced with nutrient containing 2–200 μ*M* ACC plus 10 m*M* $CoCl_2$ or ACC alone. At the end of 2 h the seedlings were encapsulated and incubated in the dark at 26°C for 1 h as described below for ozone exposure.

Ozone exposure
Following the various inhibitor and/or ethylene intermediate treatments, the seedlings were exposed to 0.6 μl l^{-1} ozone in the modified greenhouse exposure chambers (Heck *et al.* 1968) under daylight conditions (280–489 μE m^{-2} s^{-1}/27–29°C) for 2 h. Ozone was generated and monitored as described by Tingey and associates (1976). Root aeration was maintained during inhibitor treatment and stress exposure. After ozone exposure, the seedling foliage and stem were sealed in 500 ml polypropylene containers which fit into the inverted lids of the nutrient container (Fig. 1). The encapsulation container was fitted with a septum and vial containing propanolamine buffer (Tingley *et al.* 1980) to maintain CO_2 at ambient concentration (about 350 μl l^{-1}). Encapsulated seedlings were incubated at 26°C in the dark for 1 h prior to ethylene determination.

Cadmium and 2,4-D
Seedlings were placed in nutrient containing 1.0 μ*M* AVG under light conditions for 2 h. At the end of this period, the nutrient was replaced with one containing a known concentration of $CdCl_2$ or 2,4-D, and seedlings maintained for 4 h under light conditions in controlled environmental chambers. Following the toxic chemical treatment, seedlings were encapsulated and incubated for ethylene determination as described for the ozone exposure. Control plants were not treated with AVG prior to $CdCl_2$ or 2,4-D treatment.

Response time of stress ethylene
The time course of ozone-induced ethylene production was studied in the static system described above for encapsulation and accumulation of ethylene (Fig. 1), except a darkened 250-ml polypropylene container was employed for encapsulation and incubation. A 30-min exposure to 0.6 μl l^{-1} ozone was sufficient to induce a measurable ethylene response. Following exposure, the seedlings were immediately encapsulated and 1.0 ml headspace samples withdrawn at 6-min intervals over a 40-min period for ethylene determination. Samples were also taken at 60, 90, and 120 min after exposure.

Ethylene determination
Headspace air samples (1.0 ml) were withdrawn from the encapsulated seedlings with a gas-tight syringe. Ethylene concentration was determined as described by Tingey *et al.* (1978). Ethylene was detectable at concentrations of 4 nl l^{-1} in a 1.0-ml sample with a retention time of 1.5 min. CO_2 concentrations were determined chromatographically with a Carle thermal conductivity detector set at 24 mA. A Poropak Q (0.32 cm × 3.7 m) at 96°C was used for the isothermal separation of CO_2. The carrier gas was helium at a flow rate of 30 ml/min.

Statistical analysis
Earlier studies in this laboratory determined that the variance of the ethylene measurements increased with increasing ethylene concentration. Therefore, the data were converted to their respective natural logarithms to stabilize variability (Snedecor and Cochran 1968). All statistical analysis were performed on log-transformed data, but for presentation transformed back to original scale as the geometric means and standard geometric deviations.

Chemicals
L-Methionine, *S*-adenosylmethionine (Grade II) and 1-aminocyclopropane-1-carboxylic acid was purchased from Sigma Chemical Company, St. Louis. Cycloheximide (B-Grade) was from Calbiochem, San Diego. $CdCl_2$ and $CoCl_2 \cdot 6H_2O$ (reagent grade) were from J. T. Baker Chemical Company, Phillipsburg, N. J. and 2,4-dichlorophenoxyacetic acid (Esteron $99^{T.M.}$) was purchased from DOW Chemical Company, Midland, Michigan. Aminoethoxyvinylglycine (AVG) was a gift from Dr. Arthur Stempel, Hoffman-La Roche Incorporated, Nutley, New Jersey.

Results and discussion

In the present study, soybean seedlings exposed to ozone exhibited approximately a 10-fold increase in ethylene production above basal levels (Fig. 2). Ozone has been shown to induce stress ethylene production in a concentration dependent manner in detached leaves (Abeles and Abeles 1972) as well as a large number of plant species (Craker 1971, Tingey *et al.* 1976). Inclusion of AVG at various concentrations (0.01–100 μ*M*) in the hydroponic nutrient 22 h prior to ozone exposure significantly reduced stress ethylene (Fig. 2). At 10 μ*M* AVG, virtually complete inhibition (93%) was observed. The root uptake of AVG at these various concentrations did not significantly alter leaf resistance (data not shown). Thus, the inhibition of stress ethylene production was not the result of stomatal closure preventing ozone entry. Basal ethylene production remained unchanged in plants treated with AVG and not exposed to ozone. However, alterations in basal ethylene production was difficult to determine due to the limit of detectability of the system.

Addition of 1.0 μ*M* AVG 2 h prior to ozone exposure was sufficient to reduce stress ethylene production by 65%, indicating that root uptake and action of AVG was fairly rapid within the hydroponic system (Fig. 3). Subsequent studies employed AVG at 1.0 μ*M* for 2 h.

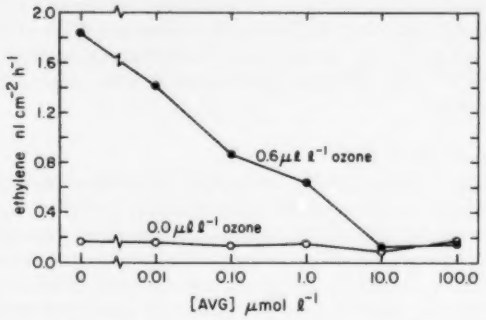

Fig. 2. Effect of various concentrations of aminoethoxyvinylglycine (AVG) exposed to ozone. AVG was added to the hydroponic media 22 h prior to the 2-h ozone exposure (1000–1200 h). Each geometric mean was based on six observations except 0 and 1.0 μ*M* AVG for which there were 10 observations each. The pooled standard geometric deviation is 1.57.

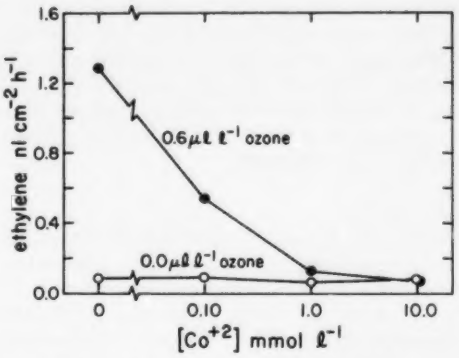

Fig. 4. Effect of various concentrations of $CoCl_2$ on stress ethylene production of soybean seedlings exposed to 0.6 μl l^{-1} ozone. $CoCl_2$ was added in nutrient solution 4 h prior to the 2-h ozone exposure (1000–1200 h). Each point is a geometric mean of six observations. The pooled standard geometric deviation is 1.33.

The effective concentration of AVG was low, similar to that required to inhibit ethylene synthesis in isolated vegetative and fruit tissue (Adams and Yang 1979, Yu and Yang 1979, Jones and Kende 1979, Yu and Yang 1980). However, the response time for inhibition was more rapid in the whole plant than that reported for the vegetative tissue (6 h). The rapid uptake and action of inhibitor insures the absence of conflicting or interfering reactions.

Cobalt is another inhibitor employed in the study of endogenous or auxin-induced ethylene biosynthesis. The proposed site of action of Co^{2+} is distinct from that of AVG: preventing the conversion of ACC to ethylene (Yu and Yang 1979). Inclusion of cobalt chloride in the hydroponic nutrient 4 h prior to ozone exposure significantly reduced the ozone-induced stress ethylene production (Fig. 4). The degree of inhibition was concentration dependent, with 1 m*M* cobalt chloride inhibiting ethylene production by 90%. Cobalt had no observable effect on basal ethylene production (Fig. 4). The root uptake of Co^{2+}, like that of AVG, did not significantly alter leaf resistance. Like AVC inhibition, the effective concentration of Co^{2+} was similar in our whole plant system to that required for isolated vegetative tissue, but again the response time of 2–4 h for the whole plant system was more rapid (Yu and Yang 1979).

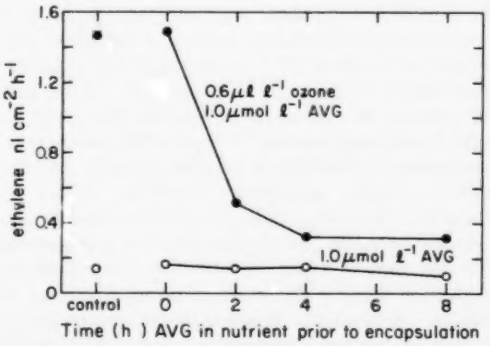

Fig. 3. Effect of aminoethoxyvinylglycine (AVG) incubation time prior to encapsulation on stress ethylene production in soybean seedlings. 1.0 μ*M* AVG added to the hydroponic media after ozone exposure, at the time of encapsulation and dark incubation (0 time); at the time of ozone exposure (2 h); 2 h prior to ozone exposure (4 h); and 6 h prior to ozone exposure (8 h). Control contains no added AVG. Each point is a geometric mean of 12 observations except the 0-h and 8-h time, which contain six observations each. The pooled standard geometric deviation is 1.51.

The proposed intermediates of endogenous ethylene biosynthesis were tested for their ability to overcome AVG and Co^{2+} inhibition of ozone-induced ethylene synthesis. Table 1 presents the results from the inclusion of these biosynthetic intermediates in the hydroponic system following a 2-h treatment with 1.0 μ*M* AVG with and without exposure to ozone. Methionine at 1.0 m*M* did not significantly reverse the inhibition of stress ethylene production by AVG. In addition, the length of time methionine was present (1, 2, 4, and 24 h) in the nutrient media did not alter the inhibition by AVG (data not shown). Similarly, increasing the concentration of methionine (1 m*M* – 100 m*M*) had no effect on reversing this inhibition (data not shown). Although methionine did not reverse AVG inhibition, the addition of 1.0 m*M* methionine to seedlings not previously treated with AVG did result in an increased stress ethylene production following ozone exposure (Tab. 1).

Leiberman and associates (1975) suggested that inhibition of AVG was irreversible and not competitive. However, Boller *et al.* (1979) demonstrated that AVG competitively inhibited the conversion of SAM to ACC

Tab. 1. The effect of AVG and ethylene biosynthetic intermediates on ozone-induced stress ethylene production. For 'AVG and intermediate' treatment 1.0 μ*M* AVG was added in fresh nutrient to the hydroponic system 2 h prior to ozone exposure. At the time of exposure, the nutrient was replaced with either fresh nutrient ('AVG' only) or nutrient containing indicated concentrations of intermediates ('AVG and intermediate'). For plants treated with intermediates only, fresh nutrient containing the appropriate intermediate was added at the time of ozone exposure with no prior AVG treatment. 'Control' plants received neither AVG nor intermediate. N: Number of observations per treatment mean. ND: No determination.

Intermediate m*M*	Ozone μl l^{-1}	N	Ethylene nl cm^{-2} h^{-1} Geometric mean (% Inhibition) Control	Inter-mediate	AVG 1.0 μ*M*	AVG, 1.0 μ*M* and intermediate	Standard Geometric Deviation
Methionine 1.0	0.0	9	0.108	0.120	0.167	0.144	1.47
	0.6	9	1.169	1.881[1]	0.452 (61%)[1]	0.517 (56%)	
SAM 1.0	0.0	4	0.086	ND	0.084	0.092	1.27
	0.6	4	1.418	ND	0.350 (75%)[1]	0.586 (59%)[2]	
ACC 0.5	0.0	6	0.191	8.876	0.252	7.301	1.34
	0.6	6	1.548	9.538[1]	0.454 (71%)[1]	9.426[2]	

[1] Significant difference (t-test, $\alpha = 0.05$) compared to control plants.
[2] Significant difference (t-test, $\alpha = 0.05$) compared to AVG-treated plants.

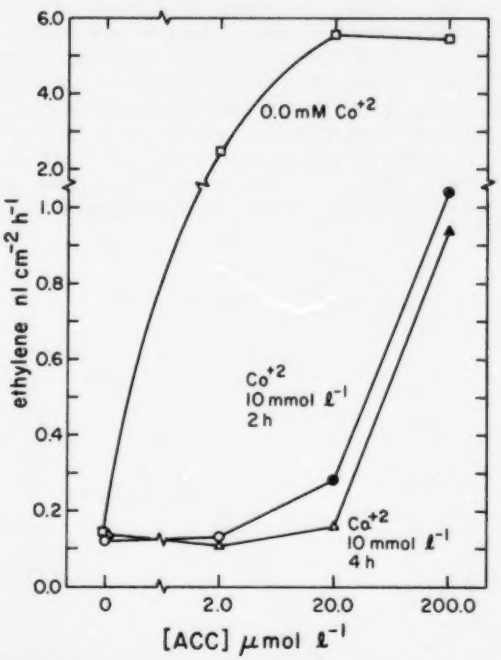

Fig. 5. Effect of various concentrations of 1-aminocyclopropane-1-carboxylic acid (ACC) on $CoCl_2$ inhibition of ethylene production in soybean seedlings. 10 m*M* $CoCl_2$ was added to the nutrient solution for a period of 2 h in the light at 26°C. At the end of this period nutrient solution containing the ethylene inhibitor was replaced with nutrient containing ACC plus $CoCl_2$(△-△) or ACC alone (○-○) for a period of 2 h in the light at 26°C. Control plants were incubated 2 h with ACC only (□-□). Each point is a geometric mean of four observations. Pooled standard geometric deviation of control plants is 1.18. Pooled standard geometric deviation of ACC and cobalt treated plants is 1.79; solid symbols, denote significant difference at $\alpha = 0.05$ compared to plants not treated with ACC.

and was reversible with addition of SAM in an isolated enzyme system from tomato fruit. The addition of 1.0 m*M* SAM to the hydroponic nutrient media did result in a slight, yet significant, reversal of AVG inhibition (Tab. 1). With AVG present, ethylene production was reduced by 75% following exposure to ozone; if 1.0 m*M* SAM was added to the AVG-inhibited plant at the time of ozone exposure, a 59% inhibition of ethylene production was observed. Increasing concentrations of SAM, however, were not employed to investigate further the suggested competitive inhibition of AVG.

The addition of the intermediate ACC results in a large increase in stress ethylene production apparently overcoming the inhibition of AVG as expected (Tab. 1). Indeed, the amount of ozone-induced stress ethylene produced by AVG plus ACC is similar to that produced by addition of ACC alone. An ACC-dependent ethylene production was demonstrated in the solution-grown soybean by induction of ethylene synthesis independent of the ozone stress (Tab. 1; Fig. 5). This ACC-dependent ethylene production was described earlier in vegetative tissue (Yu and Yang 1979, Bradford and Yang 1979) and more recently in solution-grown tomatoes (Bradford *et al.* 1980). The concentration dependence of the ACC-dependent ethylene synthesis in the soybeans is shown in Figure 5 with 2.0–200 μ*M* ACC. The ability of ACC to overcome Co^{2+} inhibition of basal ethylene production in the hydroponically-grown soybean was apparently concentration-dependent, with no relief of inhibition at 2.0 μ*M* ACC and significant reversal of inhibition at 200 μ*M* (Fig. 5). Yu and Yang (1979) demonstrated the concentration dependence of Co^{2+} on the inhibition of ACC-dependent ethylene production; however, the converse, the concentration dependence of ACC on reversing Co^{2+} inhibition of ethylene production, was not

Tab. 2. Inhibition of chemically-induced stress ethylene production by AVG. $CdCl_2$ or 2,4-D was added in fresh nutrient solution at the indicated concentration 4 h prior to encapsulation. Control plants were not treated with AVG. For $CdCl_2$ treatment, data reported is the geometric mean of six observations with a standard geometric deviation of 1.80. For 2,4-D treatment, data shown is the geometric mean of three observations with a standard geometric deviation of 1.84.

		Ethylene nl cm^{-2} h^{-1}		
Chemical m*M*		Control	AVG 1.0 μ*M*	Inhibition %
$CdCl_2$	2	0.1152	0.0988	14
	1.0	0.7795	0.1448	81[1]
	10.0	0.6796	0.1361	80[1]
	100.0	0.8218	0.3152	62[1]
2,4-D	0	0.0944	0.1270	–
	0.01	0.1755	0.0612	65[1]
	1.00	0.4976	0.1185	76[1]

[1] Significant inhibition of ethylene production (t-test, α = 0.05).

shown. The ethylene response was not altered by increasing the time cobalt is present in the nutrient from 2 to 4 h (Fig. 5). Inclusion of 200 μ*M* ACC in the nutrient solution of 10 m*M* cobalt-inhibited plants exposed to ozone resulted in an 88% recovery of stress ethylene production (data not shown).

The production and inhibition of stress ethylene generated in response to other types of external stimuli was also investigated in the solution-grown soybean seedlings (Tab. 2). Stress ethylene production increases 86% with increasing cadmium chloride concentration. Addition of AVG significantly reduced stress ethylene by 60 to 80%. Similar results were obtained with 2,4-D (Tab. 2). Ethylene accumulation increased 82% with increasing concentrations of 2,4-D. This production was reduced significantly, 65 to 76%, if AVG was present prior to the addition of 2,4-D. These results suggest that, like ozone-induced stress ethylene production, cadmium and 2,4-D also induced stress ethylene production, and inhibition by AVG suggest a biosynthetic pathway similar to that for basal ethylene.

The regulation of stress-induced ethylene is not as well understood as endogenous ethylene biosynthesis (c.v. Leiberman 1979). A requirement for protein synthesis in the regulation of stress ethylene biosynthesis would be suggested by a lag period preceding an increased ethylene production following stress and inhibition by protein synthesis inhibitors. Following a 30-min exposure of intact soybean seedlings to 0.6 μl l^{-1} ozone, a lag period in production of stress ethylene of 12–18 min was observed (Fig. 6). If one assumes reaction of the plants to ozone at the beginning of the 30-min ozone exposure, the lag period would be 42–48 min. Either time course of ozone-induced ethylene is relatively short compared to the 1–2 h induction period shown for Cu^{2+}-treated bean leaves (Abeles and Abeles 1972), the 2- to 4-h induction period demonstrated in Cu^{2+}-wounded mung bean hypocotyl (Yu and Yang 1980) or the 36-h time period for anaerobically-induced stress ethylene (Bradford and Yang 1980). A fairly rapid stress ethylene induction period of 26 min was demonstrated in mechanically wounded pea stem segments (Saltveit and Dilley 1978).

In addition to the observations on the time course of stress ethylene production, the inhibition by cycloheximide of stress ethylene production in vegetative tissue has suggested a requirement for protein synthesis in regulation of stress ethylene (Abeles and Abeles 1972, Saltveit and Dilley 1979, Yu and Yang 1980). Yu and Yang (1980) demonstrated the cycloheximide inhibition of ACC synthase activity as well as ACC and ethylene in Cu^{2+}-treated mung bean hypocotyl. In our studies, including 1.0 μ*M* cycloheximide in the hydroponic nutrient of actively growing seedlings 4 h before exposure to ozone reduced stress ethylene production 42–48% (Tab. 3). Incubation of the seedling with cycloheximide for 6 or 21 h prior to exposure did not significantly increase the degree of inhibition of stress ethylene (data not shown). Leiberman and associates (1975) observed a similar incomplete inhibition of basal ethylene with 10 μ*M* cycloheximide for 24 h in apple tissue. The lack of complete inhibition could be due to: 1) the low concentration of cycloheximide employed (1.0 μ*M*), however concentrations above 3.5 μ*M* were shown to effect respiration in beet tissue (Ellis and MacDonald 1970); 2) uptake of cycloheximide by the

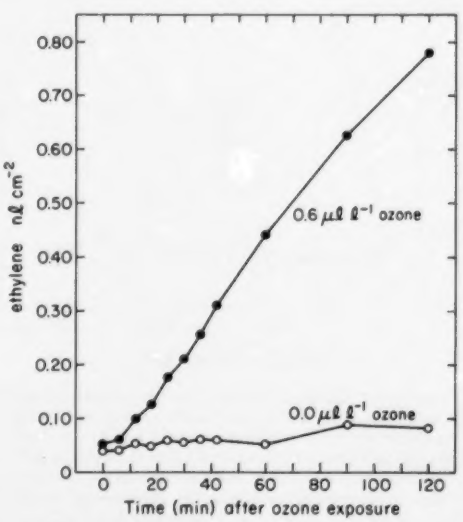

Fig. 6. Stress ethylene production by soybeans in hydroponic culture following a 30-min exposure to 0.6 μl l^{-1} ozone. Each point is a geometric mean of 12 observations at each time for ozone-exposed and seven observations at each time for non-exposed plants. Pooled standard geometric deviation is 1.42.

Tab. 3. Inhibition of stress ethylene production with cycloheximide. Cycloheximide (1.0 μM) was added in fresh nutrient to the hydroponic system 4 h prior to a 2-h ozone exposure. At 2 h, 6 h and 19 h, 1.0 ml samples were withdrawn for ethylene determination. Percent inhibition by cycloheximide was determined by comparison to control plants which were not treated with cycloheximide. The data reported represent a geometric mean of three observations per treatment. The pooled geometric standard deviation is 1.47.

Ozone $\mu l\ l^{-1}$	Cycloheximide µmol	Ethylene nl cm^{-2} Geometric mean (% Inhibition)		
		2 h	6 h	19 h
0	0	0.1084	0.1250	0.2439
	1.0	0.1129 (+3%)	0.1545 (+23%)	0.2445 (0%)
0.6	0	0.7818	1.1099	1.8669
	1.0	0.4191 (46%)	0.5749 (48%)	1.0899 (42%)

roots; 3) cycloheximide stimulation of ethylene production (Abeles and Abeles 1972); 4) multiple sites of ethylene production within the cell independent of cytoplasmic protein synthesis; or 5) the possibility that not all the stress ethylene produced requires protein synthesis (induction or synthesis of ACC synthase). A portion of the stress ethylene production could be from release of compartmentalized ethylene as described by Jerrie and coworkers (1979); however this pool would be small (3–7%) based on our studies with AVG and Co^{2+} inhibition.

The result of this study with inhibitors and intermediates of ethylene biosynthesis in intact plants, suggests that stress ethylene production follows the same or similar pathway as that described for endogenous or auxin-induced ethylene biosynthesis in isolated tissues. This study corroborates the earlier work of Abeles and Abeles (1972) in which they suggested a possible common origin of stress and endogenous ethylene based on the conversion of ^{14}C-methionine to ^{14}C-ethylene in bean leaves. The response of intact, actively growing soybean seedlings to ethylene synthesis inhibitors, AVG and Co^{2+}, as well as cycloheximide, exhibits features similar to stress ethylene synthesis recently described in isolated vegetative and fruit tissue following mechanical wounding and Cu^{2+} treatment (Saltveit and Dilley 1978, 1979; Yu and Yang 1980), and anaerobically-stressed tomato plants (Bradford *et al.* 1980). A fairly short lag period (12–48 min) preceded the ozone-induced stress ethylene production by the intact seedlings. The induction period, as well as the incomplete inhibition by cycloheximide, suggest the possibility that in the whole plant system, stress ethylene production is not totally dependent upon protein synthesis. Further studies are required to substantiate these suggestions on the regulation of ozone-induced stress ethylene production.

References

Abeles, F. B. 1973. Ethylene in Plant Biology. – Academic Press, New York. ISBN 0-12-041450-3.

Abeles, A. L. & Abeles, F. B. 1972. Biochemical pathways of stress-induced ethylene. – Plant Physiol. 50: 496–498.

Adams, D. O. & Yang, S. F. 1979. Ethylene biosynthesis: Identification of l-aminocyclopropane-l-carboxylic acid as an intermediate in the conversion of methionine to ethylene. – Proc. Natl. Acad. Sci. USA 76: 170–174.

Boller, T., Herner, R. C. & Kende, H. 1979. Enzymatic formation of an ethylene precursor, l-aminocyclopropane-l-carboxylic acid. – Planta 145: 293–303.

Bradford, K. J. & Yang, S. F. 1980. Xylem transport of l-aminocyclopropane-l-carboxylic acid, an ethylene precursor, in waterlogged tomato plants. – Plant Physiol. 65: 322–326.

– Hsiao, T. C. & Yang, S. F. 1980. Root synthesis of l-aminocyclopropane-l-carboxylic acid (ACC): Role in stomatal behavior and effects of ethylene biosynthesis inhibitors. – Plant Physiol. 65: suppl. 40.

Craker, L. E. 1971. Ethylene production from ozone injured plants. – Environ. Pollut. 1: 299–304.

Downs, R. J. & Hellmers, H. 1975. Environment and the Experimental Control of Plant Growth. – Academic Press, New York. ISBN 0-12-221450-1.

Ellis, R. J. & MacDonald, I. R. 1970. Specificity of cycloheximide in higher plant systems. – Plant Physiol. 49: 227–232.

Heck, W. W., Dunning, J. A. & Johnson, H. 1968. Design of a simple plant exposure chamber. – DHEW, National Center of Air Pollution Control Publ. ATPD-68-6. Washington, D.C.

Jerrie, P. H., Shaari, A. R. & Hall, M. A. 1979. The compartmentation of ethylene in developing cotyledons of Phaseolus vulgaris L. – Planta 144: 503–507.

Jones, J. F. & Kende, H. 1979. Auxin-induced ethylene biosynthesis in subapical stem sections of etiolated seedlings of Pisum sativum L. – *Ibid.* 146: 649–656.

Kende, H. & Hanson, A. D. 1976. Relationship between ethylene evolution and senescence in Morning-Glory flower tissue. – Plant Physiol. 57: 523–527.

Leiberman, M. 1979. Biosynthesis and action of ethylene. – Annu. Rev. Plant Physiol. 30: 553–591.

– Kunishi, A. T. & Owens, L. D. 1975. Specific inhibitors of ethylene production as retardants of the ripening process in fruits. – *In* Facteurs et Régulations de la Maturation des Fruits. – Colloques Int. C.N.R.S. No. 238. Paris, 1974, pp. 161–170.

Saltveit, M. E., Jr. & Dilley, D. R. 1978. Rapidly induced wound ethylene from exercised segments of etiolated Pisum sativum L., cv. Alaska. I. Characterization of the response. – Plant Physiol. 61: 447–450.
– – 1979. Studies of rapidly induced wound ethylene synthesis by excised sections of etiolated Pisum sativum L., cv. Alaska. IV. Requirement of a water-soluble heat-stabile factor. – Plant Physiol. 64: 417–420.
Snedecor, G. W. & Cochran, W. G. 1957. Statistical Methods. – 6th ed. Iowa State University Press, Ames. ISBN 0-8138-1560-6.
Tingey, D. T. 1980. Stress ethylene production: A measure of plant response to stress. – HortScience 15: 630–633.
– Standley, C. & Field, R. W. 1976. Stress ethylene evolution: A measure of ozone effects on plants. – Atmos. Environ. 10: 1–6.
– Pettit, N. & Bard, L. 1978. Effect of chlorine on stress ethylene production. – Environ. Exp. Bot. 18: 61–66.
– Raba, R. M. & Burns, W. F. 1980. Diethanolamine-carbon dioxide buffer produces ethylene. – J. Exp. Bot. 31: 347–351.
Yang, S. F. & Pratt, H. K. 1978. The physiology of ethylene in wounded plant tissue. – *In* Biochemistry of Wounded Plant Tissue (G. Kahl, ed.), pp. 596–622. Walter de Gruyter, Berlin. ISBN 3-11-006801-x.
Yu, Y-B. & Yang, S. F. 1979. Auxin-induced ethylene production and its inhibition by aminoethoxyvinylglycine and cobalt ion. – Plant Physiol. 64: 1074–1077.
– – 1980. Biosynthesis of wound ethylene. – *Ibid.* 66: 281–285.

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